**Supplementary Material Available:** Experimental details for the synthesis of 1 and NMR spectra of the methylene protons of the undeuterated epoxide, the deuterated epoxide from the three different reaction times, and simulations of mixtures (9 pages). Ordering information is given on any current masthead page.

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## Atomic Structure of the Rapamycin Human Immunophilin FKBP-12 Complex

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Complexes of immunophilins with immunosuppressive drugs interfere with a variety of signal transduction pathways in the cytoplasm of the cell.[1-3] Rapamycin[4] (1) is a high affinity ligand ($K_d$ = 0.2 nM)[2] to the immunophilin FKBP-12[5-7] and appears to be a general and potent antiproliferative agent.[1] The pleiotropic actions of rapamycin on growth factor receptor signaling pathways have elevated this compound to a high status as a probe of signaling mechanisms. Although the precise details have yet to be elucidated, the *complex* of human FKBP-12 and rapamycin has been shown by genetic methods to function as the inhibitory agent.[8] Herein we report the three-dimensional structure of the complex of human FKBP-12 and rapamycin, determined to 1.7-Å resolution

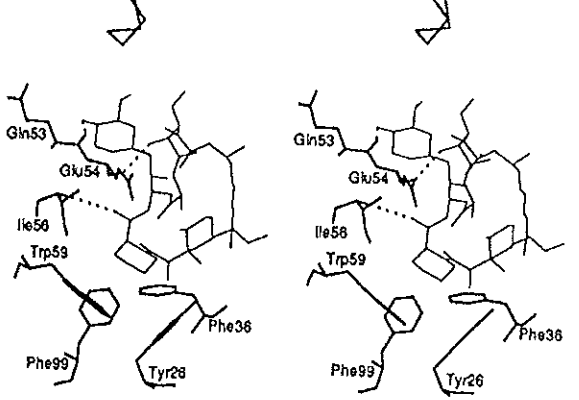
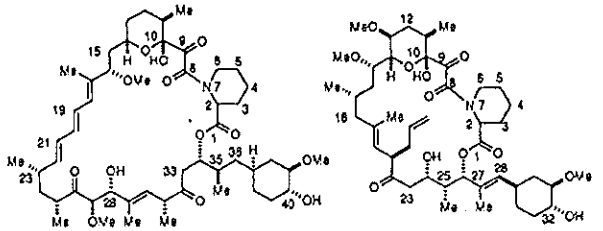



**Figure 1.** (a, top) A stereoview of the α-carbon tracing of FKBP-12 and rapamycin. The N- and C-terminal α-carbons are labeled. (b, bottom) A stereodrawing of the binding pocket showing all of the bound rapamycin molecule and selected FKBP-12 residues.

by X-ray crystallographic techniques.[9] This structure provides a framework to interpret the effects of structural perturbation of either rapamycin or human FKBP-12 on signal transduction pathways.



1 - Rapamycin 2 - FK506

As shown in Figure 1, the protein component of the FKBP-12/rapamycin complex forms a five-stranded antiparallel β-sheet

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(9) Crystals of the FKBP-12/rapamycin complex were grown from solutions containing 10 mg/mL of protein complex, 300 mM ammonium sulfate, and 100 mM phosphate at pH 6.0 using the hanging drop method at room temperature. The space group is $P2_12_12_1$ with $a$ = 45.42 Å, $b$ = 49.16 Å, $c$ = 54.74 Å, and one molecule in the asymmetric unit. Data were measured using a SanDiego Multiwire Systems Mark II detector and a rotating anode source to 1.7-Å resolution. A total of 81 484 reflections were measured (12 991 unique, 93% complete, $R_{sym}$ = 0.056, 10 633 with $F \geq 3\sigma$) from two crystals. The structure was solved using the molecular replacement method with a search model composed of the protein component of the FKBP-12/FK506 complex and the MERLOT program system.[10] The structure was refined with X-PLOR[11] using least-squares minimization by conjugate gradients where the stereochemical restraints used in ligand refinement were restricted to terms for bond lengths, bond angles, and improper dihedral angles (for planar $sp^2$ carbons and chiral centers). The conformation of bound rapamycin was determined unambiguously from well-defined electron density in $2F_o-F_c$ maps. The $R$ factor for the current model, including FKBP-12, rapamycin, and 85 water molecules, is 0.165. All main chain atoms, all buried side-chain atoms, and all ligand atoms are well-defined in the final $2F_o - F_c$ electron density map. The root-mean-square deviations of bond lengths and bond angles from their ideal values are 0.01 Å and 2.8°, respectively.

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wrapping with a right-handed twist around a short α-helix—the same folding topology found in the complex of FKBP-12 with FK506[12] and in uncomplexed FKBP-12.[13,14] The root-mean-square (rms) deviations of α-carbons, backbone atoms, and all protein atoms between FKBP-12 complexed with rapamycin and FKBP-12 complexed with FK506 are 0.67, 0.67, and 1.51 Å, respectively. Only one region, involving residues 31–34 in the loop between strands 4 and 5 of the β-sheet structure, adopts a different main chain conformation. These residues are not involved in protein–ligand interactions, but may be important recognition features of the complex.

Rapamycin binds in a cavity between the β-sheet and α-helix with the pipecolinyl ring deeply buried in the protein (Figure 1a). The protein–ligand interface involves atoms from the pyranose ring through the C28 hydroxyl, with the remainder, including the C17-C22 triene, exposed. The C1 ester, the pipecolinyl ring, the C8 and C9 carbonyls, and the pyranose ring adopt a conformation that is superimposable with the same groups in the FKBP-12/FK506 complex.[12] Three hydrogen bonds between this region and FKBP-12 (Ile-56 NH to C1 carbonyl, Tyr-82 hydroxyl to C8 carbonyl, and Asp-37 carboxylate to C10 hydroxyl) and a C9 carbonyl binding pocket involving C–H···O interactions with ε-hydrogens from Tyr-26, Phe-36, and Phe-99 are also identical with those found in the complex with FK506, thus confirming the identical binding roles of the common structural elements[15] in the two immunosuppressant ligands.

Two additional hydrogen bonds are involved in rapamycin binding to FKBP-12 (Figure 1b). The first is from Glu-54 main chain carbonyl to C28 hydroxyl, which along with the Ile-56 NH to C1 carbonyl–hydrogen bond may mimic the interaction of the dipeptide portion of a natural substrate with FKBP-12. It has been noted that the pyranose–pipecolinyl region also mimics a dipeptide,[16] making rapamycin, like FK506, a possible example of an extended peptide mimic. This hydrogen bond is analogous to the one from Glu-54 main chain carbonyl to C24 hydroxyl found in the FKBP-12/FK506 complex.[12] The second hydrogen bond is from Gln-53 main chain carbonyl to the C40 hydroxyl. In the rapamycin complex the cyclohexyl group (C35–C42) is bound to the protein through this hydrogen bond, while the FK506 complex has no such cyclohexyl–protein interaction. FK506's (2) C27–C28 double bond restricts the orientations of the cyclohexane while in rapamycin (1) the cyclohexyl ring can swing about the C35–C36 bond to form a Gln-53 carbonyl to C40 hydroxyl hydrogen bond.

The conformation of bound rapamycin is virtually identical with that seen in the free, crystalline state,[4] with an rms difference of 0.49 Å. Unlike FK506, which undergoes a cis to trans isomerization of the amide bond accompanied by a dramatic change in overall conformation on binding to FKBP-12,[12] rapamycin possesses a high degree of structural preorganization for binding. This preorganization, along with the anchoring of the cyclohexyl group, may explain the twofold higher affinity ($K_d$ = 0.2 nM) of rapamycin for FKBP-12 compared to FK506 ($K_d$ = 0.4 nM).[2]

The view of the FKBP-12/rapamycin complex as the biological effector in immunosuppressive function requires a focus on the complex as a whole—in particular the exposed regions of bound rapamycin and the FKBP-12 loops flanking the binding site. The likely role of FKBP-12 and other FKBP's in the disruption of signal transduction in T-cells is to present rapamycin (or FK506) to as yet unknown biological acceptors, or partner proteins. The FKBP-12/rapamycin complex described may be best viewed in this context as the ligand, now known at atomic resolution, to a partner protein involved in cytoplasmic signal transduction.

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## Self-Assembling, Alkali-Metal-Complexing Nickel Salicylaldimine Complexes

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Nature often achieves biological function in large molecules that are shaped and ordered by various feeble forces such as hydrogen bonding, salt-bridge formation, π-stacking, etc. We[1] and others[2] have been interested in this phenomenon especially from the perspective of developing relatively small molecular hosts that can assemble, organize, and bind. This phenomenon has two manifestations that should be distinguished, however. On the one hand, there are those that self-assemble to bind with little structural change.[3] On the other, there are hosts such as carboxypeptidase A that undergo significant structural change ("induced-fit system") when a guest is bound.[4] A model in the former category was devised by Reinhoudt et al., who used a macrocyclic salen–polyether–$UO_2$ complexes to afford a binding site for urea.[5] We now report an unusual nickel salicylaldimine system that was thought[6] to be in the former category but actually forms an unusual bimetallic molecular cage.

3-Hydroxysalicylaldehyde was converted into a series of 3-alkoxy-*N*-methylsalicylaldimine derivatives as previously described.[6] The side arms in the 3-position included methyl ($CH_3$, 1), 2-methoxyethyl ($CH_2CH_2OCH_3$, 2), and 2-(2-methoxyethoxy)ethyl ($CH_2CH_2OCH_2CH_2OCH_3$, 3). It is known that such aldimine systems react with nickel to form square-planar nickel(II) complexes of the $NiL_2$ variety.[7] These complexes may undergo tetrahedral–square planar equilibria if the system is sterically hindered. Some diamagnetic, square-planar complexes further associate by forming paramagnetic dimers.[8] We isolated the complex $I_2Ni$ as previously reported.[6] The combustion analysis and mass spectrum were compatible with the indicated stoichiometry. Assessment of stoichiometry in such cases by vapor pressure osmometry (VPO) has been eschewed as the results do not always accord with those of cryoscopic studies (see supplementary material).[9] Our studies using VPO indicated that $I_2Ni$

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